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OVULE AND EMBRYO OF POTAMOGETON NATANS.

CONTRIBUTIONS FROM THE HULL BOTANICAL LABORATORY. XXVIII.

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(WITH PLATES II AND III AND ONE FIGURE)

THIS investigation was begun several years ago, and was continued as time permitted. Material from a number of stations in the vicinity of Chicago was collected, and other collections have been added. From this material about seven hundred slides of microtome sections have been prepared and studied. The work was done under the direction of Professor John M. Coulter and Dr. Charles J. Chamberlain.

MATERIAL AND METHODS.

The collections were made in June, July, and August. Those made in June show some young spikes still within the cone-shaped spathe, while others are just issuing from it. Early in July the flowers open and pollination is effected, while in August the seed comes to maturity.

The spikes were cut off with a sharp scalpel and dropped at once into the 1 per cent. solution of chrom-acetic acid. After remaining in the solution from 36 to 48 hours, the material was washed in warm water, and then allowed to remain in running tap water 20 to 24 hours. After imbedding in paraffin the material was cut on a Minot microtome, the sections being from 6–13μ in thickness.

Combination stains were used for all work, cyanin and erythrosin proving most satisfactory, particularly for sporogenous tissues. The safranin gentian-violet orange G combination gave very satisfactory results for chromosomes and all phases of mitosis. The length of time sections should be left in each

stain became a matter of some experimentation. In general, 30 to 40 minutes in cyanin and 30 to 40 seconds in erythrosin gave the bests results. The use of turpentine between absolute alcohol and xylol was found to be of decided advantage in many cases.

FLOWERS AND SPIKES.

The flower consists of four similar cup-shaped sepals, four opposite stamens, and four alternate carpels. In early stages the sepals completely overlap the other organs, but later they spread sufficiently to allow the protruding styles to expose the stigmas for pollination. Each stamen branches, bearing an anther on each branch. The anthers are biloculate, and hence the group of four carpels is surrounded by sixteen pollen-sacs. The flowers are nearly sessile on the vertical spike, alternating in such a way that the spiral arrangement is clearly seen (*fig. 1*).

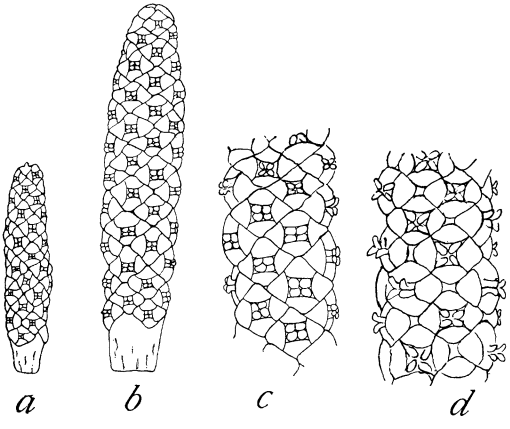


FIG. 1.—Illustrations of spikes: *a*, × $\frac{2}{3}$ size; *b*, × $\frac{4}{3}$; *c*, × $\frac{5}{3}$; *d*, × 2.

From these spikes longitudinal and transverse sections were cut. It can be seen that the first and last longitudinal sections from such a cylindrical spike would give cross-sections of stigmas, styles, and ovaries; while those cut from the center would give two rows of longitudinal sections. Cross-sections of the spike would of course give longitudinal sections of ovaries, styles, and stigmas, but in a plane at right angles to the longitudinal sections mentioned above.

THE OVULE.

The embryo sac is much more deep-seated than in many other plants, occurring in the center of the nucellus, and surrounded

on all sides by a mass of tissue from five to eight cells in depth. This deep-seated position may bring added protection of some sort, or it may have no such significance. In the earliest stages of the nucellus no differentiation of cells can be distinguished. Its growth appears to be due almost entirely to divisions of nucellar tissue other than epidermal, since after the formation of the epidermis no layers were observed cut from it by periclinal walls. In fact, the occurrence of periclinal walls in the epidermis is exceedingly rare.

The archesporial cell and its two daughter cells.—Longitudinal sections of the nucellus, about the time the primordium of the inner integument makes its appearance, show a single hypodermal cell having archesporial characters. This cell is larger than the others and has a larger nucleus, and its protoplasm is usually less dense (*fig. 1*). When this hypodermal cell is barely distinguishable from the surrounding tissue, it divides by a periclinal wall. The outer cell is the so-called "tapetal cell" (*figs. 2, 3*), from the progeny of which an extensive region of sterile tissue is subsequently formed. The inner cell is the primary sporogenous cell or megaspore mother cell, its sporogenous character being plainly shown by the rapid changes which soon take place in both its nucleus and cytoplasm.

The sterile tissue.—The subsequent history of the tapetal cell is as follows. The increase in size is followed at once by a periclinal division (*fig. 4*), and this by an anticlinal wall (*fig. 5*). Doubtless an anticlinal wall follows in each of these cells, making a plate of four cells lying next to the epidermis. Wiegand[1] says that the anticlinal walls may precede or follow the periclinals, usually preceding, but this was not verified in *P. natans*. Anticlinal walls now follow in the second layer, thus completing a two-layered tapetum. In *fig. 7* a three-layered tapetum is shown; in *fig. 13* one that is five-layered. The process continues until in some cases eight layers lie between the embryo sac and the epidermal layer. As mentioned before, the embryo sac now lies in the very heart of the nucellus.

[1] WIEGAND KARL M.: The development of the embryo sac in some monocotyledonous plants. BOT. GAZ. **30**: 25–47. *pls. 6–7.* 1900.

The primary sporogenous cell.—At the same time the primary sporogenous cell is undergoing important changes. It increases very much in size, its contents becoming less and less dense, until in some cases vacuoles make their appearance (*fig. 6*). The nucleus also increases in size, and is often surrounded by an area of hyaline cytoplasm (kinoplasm), while radiating strands of granular protoplasm extend from the nucleolus to the periphery of nucleus (*figs. 2, 3*). The chromatin of the nucleus now collects in a closely compacted mass, with a dense non-granular nucleolus by its side (*figs. 4, 5, 6*), the cell having passed into the synapsis stage so characteristic of mother cells.

That the development of the primary sporogenous cell has been simultaneous with the formation of tapetal tissue is shown by the fact that in many cases the former cell has been found in early synapsis with only a two-layered tapetum (*fig. 4*). As the nucleus of the mother cell passes to the spirem stage, the chromatin threads are usually more on one side than on the other. The nucleoli never appeared granular, though often one to several very large granules or black grains could be seen. The cytoplasm was never uniform in appearance, at times having a reticulated appearance (*fig. 5*), and again a stringy appearance (*fig. 6*).

It may be stated that if the manipulation of the stains has been fortunate, one finds in all phases of synapsis an excellent illustration of the value of cyanin and erythrosin as differential stains, the chromatin being always a deep red and the nucleolus a deep blue. There was in the case of Potamogeton no difference in the way in which the stains were taken up by nuclei of the megaspore and microspore mother cells, thousands of the latter showing the highly erythrophilous chromatin and the highly cyanophilous nucleoli seen in the former.

Development of potential megaspores.—It was some time before any positive evidence of an axial row could be found; but the presence of a cap of densely staining protoplasm at the micropylar end of the sac was a strong indication of its presence.

Wiegand[2] demonstrated a two-celled row for *P. foliosus*, but found the fate of the two cells somewhat unusual. He says, "the first division of the archesporial cell is immediately followed by a second nuclear division in each of the daughter cells, but without the formation of a wall between the two nuclei. The upper of these two cells soon perishes, while the lower goes on through various phases forming at last the complete embryo sac."

In *P. natans* some irregularity appears in the number of cells of the axial row, but it is always more than two, brought about by the division of one or both daughter cells of the primary sporogenous cell. The early stages of the division of the sporogenous cell are shown in *figs. 4–7*. In the stage shown in *fig. 8*, the chromosomes have passed to the poles, the spindle fibers are still intact, and the cell plate is clearly marked. A spindle is also formed in each daughter cell (*fig. 9*). It is at this point that the irregularity mentioned above appears. That a spindle is not always formed in each cell may be inferred from *fig. 11*, where the lower cell shows by its size, age, and nucleus that it is the functioning megaspore. The middle cell, its sister cell, is being resorbed along with the upper cell. The spindle shown in *fig. 10* indicates that the two resulting potential megaspores will lie side by side. The preparations referred to thus far do not show any completed four-celled axial row, but they do show several three-celled rows, with spindles to indicate four potential megaspores. The spindle in the upper cell of the axial row, in both cases shown, is transverse rather than longitudinal.

The germination of the megaspore.—The divisions leading to the ante-fertilization stage of the gametophyte appeared perfectly regular and normal, although Wiegand[3] states that in *P. pauciflorus* both egg-apparatus and antipodals were somewhat abnormal. He says, "although the normal number of cells in each was present, they were formed irregularly. The polar nucleus and

[2] WIEGAND, KARL M.: *loc. cit.* 32.

[3] WIEGAND, KARL M.: Notes on the embryology of Potamogeton. BOT. GAZ. 25: 116–117. 1888.

first and second synergids seem to have been cut off successively from the mother nucleus of the egg. The synergids disappear almost immediately. A similar irregularity was found in the antipodals." This could not be verified in *P. natans*, in which the process seems quite normal (*figs. 13, 14, 17*). At this time there is considerable plasmolysis even when great care is taken in the fixing and subsequent processes (*figs. 15, 16, 17*). In *fig. 14* traces of the first spindle of the megaspore can still be seen connecting the recently formed daughter nuclei, but there is no trace of a wall. By the growth of the sac the antipodals are left in a small pocket, and are of short duration (*figs. 16, 17*); but no traces of a wall shutting them off from the rest of the sac at any time could be found. Nor could a wall be found cutting off the egg-apparatus from the opposite end of the sac. The polar nuclei never meet in the center of the sac, but always nearer the antipodal end (*figs. 16, 17*). The endosperm develops as a parietal layer of cytoplasm in which free nuclei are imbedded, and no walls were observed in the most advanced stages studied (*figs. 19, 21d*).

THE EMBRYO.

The first division of the oospore is transverse, resulting in a large vesicular suspensor cell and the first cell of the embryo proper (*fig. 18*). The three-celled embryo shown (*fig. 19*) has probably resulted from the division of the embryo cell. The single suspensor cell becomes remarkably large and vesicular, and its nucleus and nucleolus correspond in size. The first division of the terminal cell in the row of three is longitudinal (*fig. 20*), and this is followed by a second longitudinal division at right angles to the first (*fig. 21*). This quadrant stage is also shown in transverse section in *fig. 21d*. Further views of the embryo at this period are shown in *figs. 21a–21d*, which are consecutive transverse sections from such an embryo as that shown in *fig. 21*, in which figure the points at which the sections are made are lettered *a, b, c, d*. It is seen from the sections that the young embryo is cylindrical rather than flattened.

In two or three cases a very large nucleus was found near the antipodal end of the embryo sac, the embryo being in the stage just described. Its origin could not be determined, but it seems reasonable to consider it a derivative of the primary endosperm nucleus, and possibly the lower nucleus of the first division, as in *Sagittaria*[4] and *Potamogeton pauciflorus*.[5]

THE UNIVERSITY OF CHICAGO.

EXPLANATION OF PLATES II AND III.

FIG. 1. Micropylar end of nucellus, showing the archesporial cell.

FIG. 2. Primary sporogenous and tapetal cells.

FIG. 3. The same at a later stage; nucleus with radiating strands of protoplasm, and surrounded by a hyaline area.

FIG. 4. Tip of a young nucellus; inner integument and primordium of outer integument; sporogenous cell with nucleus in early synapsis stage; chromatin massed, dense (deeply erythophilous); nucleolus non-granular (deeply cyanophilous); two-layered tapetum.

FIG. 5. Primary sporogenous cell; nucleus in synapsis; a two-layered tapetum with an anticlinal wall recently formed.

FIG. 6. Same with two-layered tapetum; nucleus in synapsis.

FIG. 7. Same with three-layered tapetum.

FIG. 8. Same with spindle and cell-plate; epidermis with double layer but taking no part in tapetal structure.

FIG. 9. Same with two spindles; upper one nearly transverse; beginning of axial row.

FIG. 10. Axial row showing two potential megaspores, and the upper daughter cell with a spindle.

FIG. 11. Functioning megaspore more advanced, but no sign of further division in either middle or upper cells.

FIG. 12. Functioning megaspore resorbing the potential megaspores.

FIG. 13. Embryo sac after the first division; daughter nuclei widely separated, with large vacuole between them; six-layered tapetum.

FIG. 14. Embryo sac after second division; remnants of the spindle between the nuclei at antipodal end of sac; eight-layered tapetum not all shown.

FIG. 15. Micropylar end of embryo sac, showing cells of the egg-apparatus; from section adjacent to *fig. 16*.

[4] SCHAFFNER, JOHN H.: Contribution to the life history of *Sagittaria variabilis*. BOT. GAZ. **23**: 252–273. *pls. 20–26*. 1897.

[5] WIEGAND, KARL M.: BOT. GAZ. **25**: 117. 1898.

FIG. 16. Antipodal end of embryo sac; antipodals in pocket, and polar nuclei meeting in lower end of sac; from section adjacent to *fig. 15*.

Fig. 17. Embryo sac after third division, showing the eight-celled stage; synergids with vacuoles, egg cell beneath; antipodals in pocket, two with nuclei, remnant only of the third one; polar nuclei meeting below the center.

FIG. 18. A two-celled embryo, showing the large vesicular cell and first cell of embryo proper.

FIG. 19. A three-celled embryo imbedded in endosperm and suspended from the large vesicular cell; endosperm of free nuclei embedded in cytoplasm; each nucleus with two nucleoli.

FIG. 20. A four-celled embryo; the end cell divided longitudinally; very large vesicular cell with correspondingly large nucleus and nucleolus.

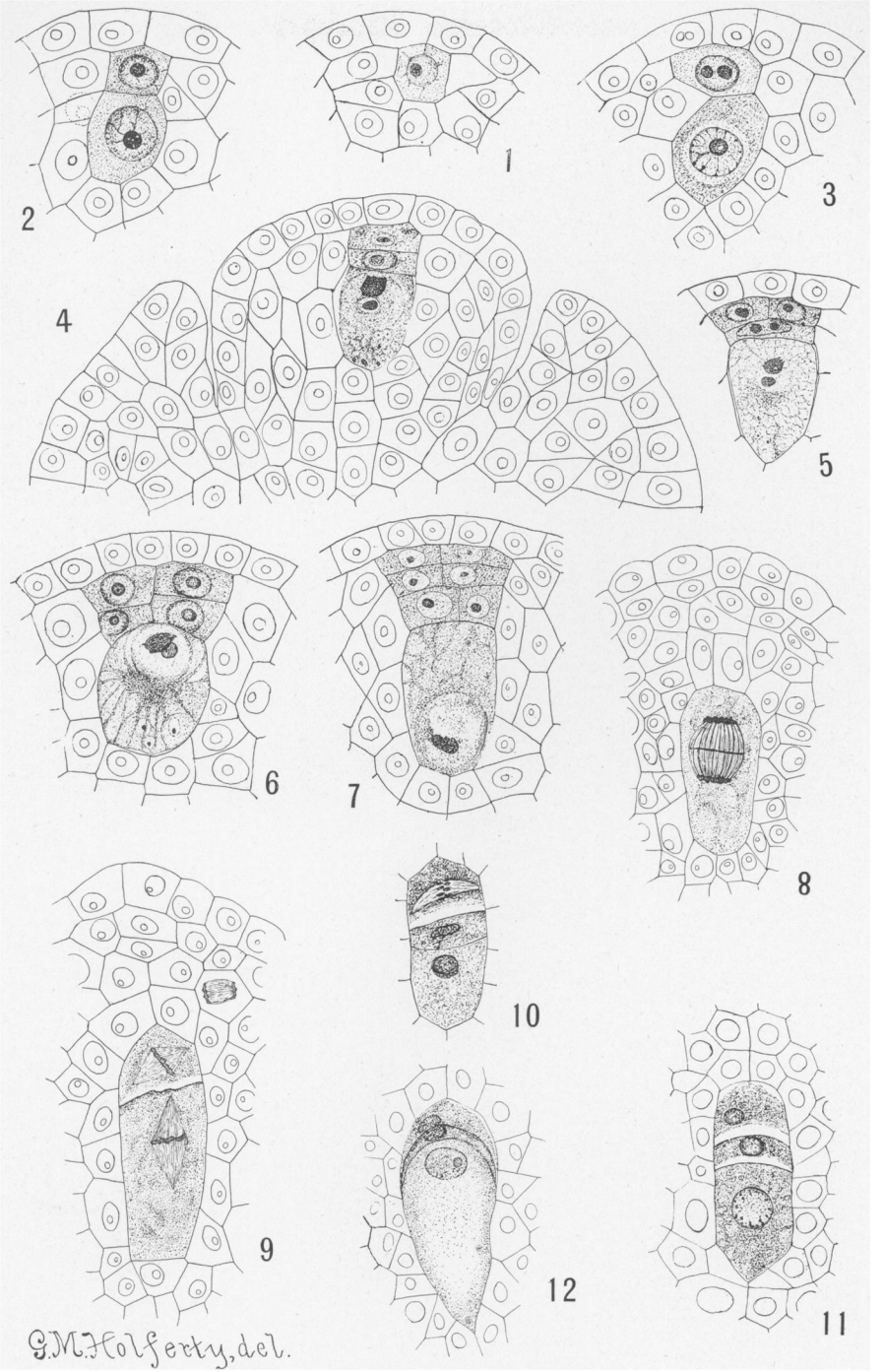
FIG. 21. An eight-celled embryo; end-cell divided into four cells; very large vesicular cell; letters denote points of cross-sections shown in following figures.

FIG. 21*d*. Cross-section of end cell through *d* in *fig. 21*.

FIG. 21*c*. Cross-section through *c* in *fig. 21*.

FIG. 21*b*. Cross-section through *b* in *fig. 21*.

FIG. 21*a*. Cross-section through *a* in *fig. 21*.



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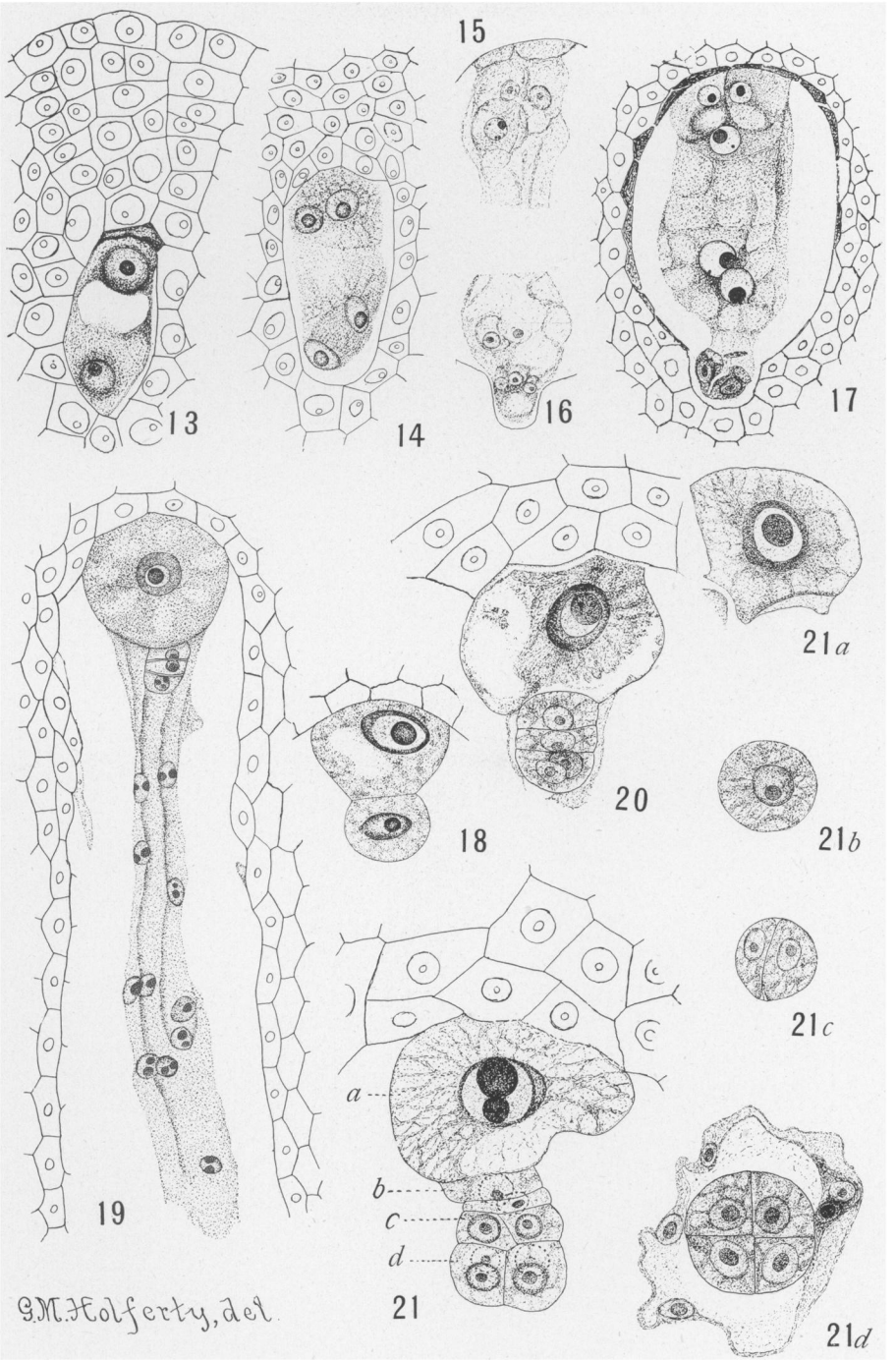

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